LSRFortessa User Guide (N.C. 03.044)

Booking

You have to book the LSRFortessa with your user name on the webpage:
https://ppms.eu/lmu/start/
Please activate the 'HTS' option when booking for a session using the plate reader.

Problems

Any problems must be reported asap as 'Incident' logging in at the webpage
https://ppms.eu/lmu/start/

Start-up

1. Make sure that there is enough **FACSFlow** and **Waste** capacity in the fluidics cart.
2. Switch on the fluidics cart (green switch).
3. Turn on the computer and log in. **Wait** until Windows is fully booted before you switch on the instrument. Do NOT launch 'DIVA' yet.
4. Switch on the LSRFortessa (green switch on the right side; the lasers need 15-20 min to warm up). **Wait >3min!**
5. Launch the 'DIVA' software. Log in under your personal user name. Wait until the program is connected with the cytometer.
6. **Disconnect the 'vent' tubes to pressurize the fluidics system.** Pressure needs about 2 min to stabilize.
7. Do a system prime by pressing the **'Prime'** button, with the sample arm being opened and no tube loaded. **Repeat 2-3 times.**
8. Run **DI water** (Millipore) at 'high' speed for a few minutes.

Acquisition

1. Check that **the 'vent' tubes are disconnected**! Pressure needs about 2 min to stabilize.
2. Make sure to have optimized your settings using appropriate controls.
3. Hit the 'RUN' button and set the flow rate on 'low', 'medium' or 'fast'.

Acquisition Troubleshooting:

- When data looks 'weird' already on FSC/SSC or no events coming through: **'Prime'** 2-3 times. Also: check that vent tubes are disconnected and that external fluid supply is ON.
- Particularly if there are problems in PE/PE-Cy7 or APC or detection: ensure that the sensor to the waste tank is NOT tightened, it should be loose to avoid backpressure! **'Prime'** again 2-3 times.
- If specifically signals of the UV laser are strange, doublecheck that the correct filter configuration is in place! You can compare to the list pasted on the binder next to the PC. Fix the filter configuration (ask for assistance if you don't know how).
- Make sure your samples are free of clumps; **Filter** them directly before acquisition in order to prevent clogging.

Data handling / Export

- Always work with experiment templates – **NEVER** duplicate an experiment.
- After exporting your data, **delete** all unnecessary data **from FACSDiva** software each time you finish with your experiment.
- Files that remain longer than one month in the database **WILL BE DELETED** by one of the responsible persons! Data storage has significant negative impact on the speed and stability of FACSDiva software.

Cleaning after yourself / between users

1. Run **FACSClean** at 'high' speed on the LSRFortessa (needs >3ml in tube) for
   1 min with arm to the side, and
   5 min with support arm under the tube
2. Repeat steps under 1 with DI water (Millipore): **1 min + 5 min**
   For this 5min wash with water: open the 'Shared View' folder at the bottom of the Experiment Explorer, open the Clean Check Experiment, and create a new tube named with your initials and date for recording the 5min water run.
3. Switch the machine to STNDBY, leaving the water tube on the SIP.
4. **Connect the 'vent' tubes to depressurize the table top tank.**
5. **Bring the filter configuration back to Default!**

Last User of the day

1. Run **FACSClean** at 'high' speed on the LSRFortessa (needs >3ml in tube) for
   1 min with support arm to the side, followed by
   5 min with support arm under the tube
2. Repeat steps under 1 with **DI water** (Millipore): **1 min + 5 min**
   For this 5min wash with water: open the 'Shared View' folder at the bottom of the Experiment Explorer, open the Clean Check Experiment, and create a new tube named with your initials and date for recording the 5min water run.
3. Switch the machine to STNDBY, leaving the water tube on the SIP.
4. **Connect the 'vent' tubes** to depressurize the table top tank.
5. **Bring the filter configuration back to Default!**
6. Switch off the flow cytometer by pressing the green main switch on the side.
7. Switch off the fluidics cart by pressing the green button.
8. Dispose of any waste/empty containers (➔ waste room NC 03.039).

Caution

- **If sheath has to be filled up, or waste container emptied, please refer to the extra instructions pasted opposite the instrument.**
- Never switch the cytometer's main switch (green) off and back on quickly, or the other way around. Doing this will destroy the lasers.

Change of containers in the Fluidics Cart Sheath Fluid

1. Press the Alarm button on the control panel to silence the alarm.
2. Loosen the sheath sensor probe cap assembly and carefully remove the sensor probe from the sheath cubitainer, keeping it at a 45-degree angle.
3. Place the sensor probe into the probe holder on the side of the fluidics cart.
4. Load the new sheath fluid cubitainer into the left side of the fluidics cart.
5. Remove the cap from the new sheath cubitainer and retain it to use as a cap when the waste container is full.
6. Insert the sensor probe at a 45-degree angle into the sheath cubitainer and tighten the cap assembly.
7. Press **Restart** until light signal disappears**.**
8. Empty Sheath Fluid containers are kept and **recycled** to become waste containers: Label it with **"next waste"**.
9. Always **leave TWO empty FACSFlow containers** to be used as next waste containers (for Fortessa and Aurora).
   All superfluous empty containers have to be trashed: separate cardboard and plastic ➔ room NC 03.039.

Waste

1. Press the Alarm button on the control panel to silence the alarm.
2. Press the STNDBY button on the cytometer.
3. Loosen the waste cap assembly and carefully remove the sensor probe from the full waste cubitainer.
   Caution: Biohazard! All biological specimen and materials coming in contact with them are considered biohazards. Wear suitable protective clothing and gloves.
4. Remove the neck support collar and save for the next cubitainer.
5. Pour **Terralin into an empty cubitainer**. Label this cubitainer as "**WASTE",** and load it into the right side of the fluidics cart.
6. Install the neck support collar and insert the sensor probe into the waste cubitainer, but **DO NOT TIGHTEN** the cap assembly.
7. Press **Restart** until light signal disappears**.**
8. **Discard the liquid waste in the sink immediately**.
9. **Trash the waste container: separate cardboard and plastic** ➔ room NC 03.039.
   Each container is only used once as a waste container.

Fluidics Cart Troubleshooting:

- If Waste alarm is coming back on soon after you emptied the waste container, then there is likely something stuck to the sensor that makes it 'think' the waste is full.
  1. Switch off the external fluidics cart.
  2. Take out the waste sensor and wipe it dry with tissues.
  3. Reinsert (DO NOT TIGHTEN) and switch the cart back on.

  This might be necessary several times.